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510(k) Summary: Safety and Effectiveness Information for the UroVysion™ Bladder Cancer Recurrence Kit

June 27, 2001

Trade Name
Vysis™ UroVysion™ Bladder Cancer Recurrence Kit

Common or Usual Name
Fluorescence *in situ* hybridization (FISH) regents

Classification Name
Class II IVD Device

Predicate Legally Marketed Device
Bard® (Bion) BTA*stat*™ Test

Description of the Device
The UroVysion Kit is based upon fluorescence *in situ* hybridization (FISH) DNA probe technology. The UroVysion probes are fluorescently labeled nucleic acid probes for use in *in situ* hybridization assays on urine specimens fixed on slides. The UroVysion Kit consists of a 4-color, four-probe mixture of DNA probe sequences homologous to specific regions on chromosomes 3, 7, 9, and 17. The UroVysion probe mixture consists of Chromosome Enumeration Probe (CEP®) 3 SpectrumRed™, CEP 7 SpectrumGreen™, CEP 17 SpectrumAqua™, and Locus Specific Identifier (LSI®) 9p21 SpectrumGold™.

Intended Use
The UroVysion Bladder Cancer Recurrence Kit (UroVysion Kit) is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence *in situ* hybridization (FISH) in urine specimens from subjects with transitional cell carcinoma of the bladder. Results from the UroVysion Kit are intended for use as a noninvasive method for monitoring for tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with bladder cancer.

Different Technological Characteristics

Both the UroVysion Kit and the BTA*stat* test use the same specimen collection and preparation techniques in clinical practice. Thus, no new issues of safety with respect to patient care are introduced by the FISH technique; both the UroVysion Kit and the BTA*stat* test start with the same patient specimen (i.e., voided urine).

The major differences between the two tests are that they detect different substances and use different detection methods. Briefly, the UroVysion Kit uses DNA probes for specific regions on chromosomes 3, 7, 9 and 17 that bind to the target chromosomes by the DNA hybridization reaction. The actual binding mechanism of the UroVysion Kit is via specific complementary base pairing. In contrast, the BTA*stat* test is a lateral flow assay that detects the presence of bladder tumor associated antigen through antigen-specific antibodies. Also, the necessary visual interpretation of the results of the UroVysion Kit and of the BTA*stat* test is different. For the BTA*stat* test, urine is allowed to react with a colloidal gold-conjugated antibody and the results are determined qualitatively by the presence or absence of a line on the test stick. For the UroVysion Kit, the analyst visually recognizes chromosomes 3, 7 and 17, and the 9p21 locus by the fluorescent signal carried by the DNA probe mixture.

Even though the technological characteristics are different between the BTA*stat* test (antigen test) and the UroVysion test (DNA probe test), both test are intended for use to monitor for the recurrence of bladder cancer from voided urine specimens. The overall performance of the UroVysion test was demonstrated to be substantially equivalent.

Safety and effectiveness issues evaluated for the UroVysion Kit included the following: prospective, comparative methods evaluation for monitoring bladder cancer recurrence; specificity evaluation in healthy and unhealthy patients (without previous diagnosis of bladder cancer); interference assessment; and reproducibility studies.

Non-Clinical Parameters

Hybridization Efficiency

On the ProbeChek™ quality control slides run in conjunction with the clinical trials, 1.5% (4/261) of the targets failed due to lack of hybridization. These slides are prepared from cultured human bladder carcinoma (positive target) and normal lymphoblast (negative target) cell lines, and represent the best-case scenario for hybridization efficiency. Thus, under these conditions, the hybridization efficiency was found to be 98.5%, with <2% cells having no signal for any of the probes.

In a reproducibility study conducted on specimens prepared from human urine cell lines, 76 of 80 specimens yielded informative results on the first attempt. Of the 4 uninformative specimens, 3 were due to lack of hybridization. Therefore the hybridization efficiency was found to be 96.2%, based on the following definition:

% Hybridization Efficiency = 100-[hybridization failures/(informative results + hybridization failures)]*100

In a specificity study conducted on urine specimens from patients with no history of bladder cancer, 230 of 309 specimens yielded informative results on the first attempt and 18 of the uninformative results were due to lack of hybridization, resulting in a hybridization efficiency of 92.7% (see "Specificity: Technical Performance: Informative vs. Non-Informative Results" for more details). Similarly, in a clinical study conducted on urine specimens from patients with a history of bladder cancer, 175 of 251 specimens yielded informative results on the first attempt and 26 of the 76 uninformative results were due to lack of hybridization. The hybridization efficiency among these specimens was found to be 87%. Thus, under these conditions, which simulate the normal clinical practice, the hybridization efficiency was found to be ≥87% (see "Performance vs. Standard of Care: Technical Performance: Informative vs. Non-Informative Results" for more details).

Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Vysis QC protocols. A total of 42 metaphase spreads were examined sequentially by reverse DAPI banding to identify chromosomes 3, 7 and 17, and the 9p21 locus, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 42 cells examined; hybridization was limited to the intended target regions of the four probes.

Interference

Three voided urine pools (one male, one female, one male/female mix) from normal healthy volunteers were spiked with the substances listed in Table 1 and assayed with the UroVysion Kit to test for possible assay interference. Replicate samples for each urine pool were evaluated for each substance (i.e., 6 samples per substance tested); 25 consecutive cells were enumerated for each specimen. No interference was detected from any of the substances tested; results from all samples were negative (*i.e.*, <4 abnormal cells as defined in this package insert). The highest concentrations tested for each substance are shown in Table 1.

Table 1
Substances Tested for Assay Interference

Substance	Highest Concentration Tested
Possible Urine Constituents	
Albumin	1.0 g/dL
Ascorbic Acid	5 g/dL
Bilirubin (unconjugated)	2 mg/mL
Hemoglobin	100 mg/mL
IgG	10 mg/dL
Red Blood Cells (human)	1×10^6 cells/mL
White Blood Cells (human)	1×10^6 cells/mL
Sodium Chloride	730 mg/dL
Uric Acid	250 mg/dL
Caffeine	117 mg/dL
Ethanol	1% (v/v)
Nicotine	28 mg/dL
Possible Microbial Contaminants	
Candida albicans	2.5×10^{10} CFU/mL
Escherichia coli	2.5×10^{10} CFU/mL
Pseudomonas aerugenosa	2.5×10^{12} CFU/mL
Therapeutic Agents	
Acetaminophen	5.2 g/dL
Acetylsalicylic Acid	5.2 g/dL
Ampicillin	600 mg/dL
BCG	20 mg/dL
Doxorubicin-HCl	10 mg/dL
Mitomycin C	10 mg/dL
Nitrofurantoin	50 mg/dL
Phenazopyridine-HCl	200 mg/dL
Thiotepa	10 mg/dL
Trimethoprin	50 mg/dL
Preservatives	
Vysis, Inc. standard: 2% Carbowax	2% Carbowax/50% ethanol solution (33 ml urine with 17 mL preservative)
UroCor, Inc. fixative	50/50 with urine
CytoRichRed (Autocyte)	50/50 with urine
Saccamono's solution	50/50 with urine
PreservCyt solution (Cytyc)	50/50 with urine
100% Ethanol	50/50 with urine

Reproducibility

Reproducibility of Patient Samples
Conducting reproducibility studies on real patient urine specimens was not feasible, since one patient cell pellet does not yield enough cells to reasonably split the specimen between observers. Hence the reproducibility of results on the number of morphologically abnormal cells was not assessed.

Reproducibility of Bladder Carcinoma Cell Culture Specimens
To assess the reproducibility of the UroVysion assay, analyses of the signal distributions for CEP 3, CEP 7, CEP 17 and LSI 9p21 were assessed for inter-site (4) reproducibility on slides prepared from 4 different bladder carcinoma cell lines. Four specimens prepared from human bladder carcinoma cell lines with normal (one specimen) and abnormal (3 specimens) signal distribution were evaluated for CEP 3, CEP 7, CEP 17 and LSI 9p21 according to the instructions for analysis of quality control slides in this package insert (see "Interpretation of Results: Analysis of Quality Control Slides"). Each site assayed four replications of the same specimen on each of four assay days (a different specimen each day), using a single probe lot for all specimens. On each assay day, an additional "wild card" specimen was added to eliminate bias and was not included in the data analysis. Each specimen was evaluated by one observer at each site. Informative results were obtained in 95.0% (76/80) of the specimens on the first attempt. Hybridization of all replacement slides was successful.

The mean, standard deviation, and percent CV of the average number of signals for the four probes is shown in Table 2. As shown in this table, the mean number of signals for each probe varies within a narrow range. The absence of LSI 9p21 signals in specimen 2 causes a large %CV for this probe, but this specimen is still easily classified as having a loss of the 9p21 locus; in 95% of the observations on this specimen (19/20) the average number of LSI 9p21 signals was <0.2.

There were no false negative results in this study of human bladder carcinoma cell lines; all (48/48) evaluations of specimens 2, 3 and 4 (16 each) would have been classified as positive by the definition of ≥ 4 cells with gains of multiple chromosomes (3 or more signals for two or more of CEP 3, CEP 7 or CEP 17), or ≥ 12 cells with homozygous loss of 9p21 (0 LSI 9p21 signals). Of the 16 evaluations of the normal specimen, one would have been classified as positive using the above definition; this case showed 6 cells with gains of multiple chromosomes.

Table 2
Between-Site Reproducibility

Specimen	Statistics[b]	Number of Signals			
		CEP 3	CEP 7	CEP 17	LSI 9p21
1	Mean	2.21	2.12	2.14	2.19
	S.D.	0.15	0.12	0.12	0.21
	C.V. (%)	6.79%	5.52%	5.66%	9.66%
	Range	2.08-2.68	1.92-2.40	1.96-2.52	2.00-2.92
	n	16	16	16	16
2	Mean	3.95	4.31	3.42	0.03
	S.D.	0.10	0.25	0.16	0.07
	C.V. (%)	2.49%	5.76	4.76%	220.44%
	Range	3.84-4.16	3.76-4.84	3.16-3.72	0.00-0.24
	n	16	16	16	16
3	Mean	4.28	3.55	3.42	3.86
	S.D.	0.32	0.34	0.25	0.47
	C.V. (%)	7.58%	9.47%	7.21%	12.14%
	Range	3.88-5.04	3.12-4.24	3.04-3.96	3.16-4.72
	n	16	16	16	16
4	Mean	3.18	3.88	3.84	3.85
	S.D.	0.15	0.10	0.10	0.15
	C.V. (%)	4.63%	2.45%	2.70%	3.90%
	Range	2.96-3.52	3.64-4.04	3.64-4.12	3.56-4.24
	n	16	16	16	16

Specificity

Study Summary
A multi-center, prospective study was conducted to establish the specificity of the UroVysion test in urine from healthy volunteers and urology patients without prior history or clinical evidence of bladder cancer.

Technical Performance: Informative vs. Non-Informative Results
A total of 315 patient visits were conducted in conjunction with this trial, resulting in 309 usable office visits. The 6 unusable visits included one that failed to meet the study eligibility criteria, 4 with insufficient urine volume, and in 1 cases urine was not sent to the testing laboratory. FISH assay and analysis on the 309 usable office visits resulted in informative results in 230 specimens on the first attempt. Of the 79 specimens that failed to yield informative results on the first attempt, only 18 were due to hybridization failures. The hybridization efficiency for the first assay attempt was 93%. The remaining non-informative assays were the result of poor specimen quality (*e.g.*, insufficient number of cells) or technical error (*e.g.*, oil under coverslip). Repeat assays were conducted on 67 specimens; 12 of these 79 specimens had insufficient volume remaining to repeat the assay. Of the 67 repeat assays, 45 yielded informative results, leaving 34 specimens classified as "non-informative" (including 12 cases with insufficient volume for repeat assay). In summary, 89% of the cases yielded an informative result on the first or second attempt. Since several patients' health conditions fell into multiple categories, the 275 patient specimens yielding informative results represented 357 data points. The patient population is summarized by category in Table 3.

Table 3
Patient Population

Condition	# of Patients
Healthy Donors	59
Non-Smokers	*50*
Smokers	*9*
Non-GU Benign Diseases	48
Non-GU Cancer	3
GU Diseases	184
BPH	*58*
Microhematuria	*15*
Interstitial Cystitis	*11*
Inflammation/Infection: Other	*17*
STD	*2*
Other	*81*
GU Cancer (non-bladder)	61
Prostate	*58*
Renal	*3*
GU Trauma	2
Total:	357

Specificity

The overall specificity of the UroVysion test in this patient population was 93.0% (332/357). The overall specificity was calculated based on all patients and all conditions; patients with medical conditions falling in multiple categories and/or multiple conditions within the same category were counted for each individual condition. A summary of the overall specificity and the specificity by category is shown in Table 4. To eliminate the potential bias of including multiple data points for any particular patient, the specificity was also calculated on "unique cases", where each patient was counted only once, regardless of the number of medical conditions present. The specificity among the unique cases was 94.5% (260/275, Table 4).

Table 4
Summary: UroVysion Kit Specificity

Overall Specificity	93.0% (332/357)
Unique Patients	*94.5% (260/275)*
Healthy vs. Non-Healthy	
Healthy	*100% (59/59)*
Non-Healthy	*93.1 (201/216)*
Smokers vs. Non-Smokers[1]	
Smokers	*95.2% (40/42)*
Non-Smokers	*94.7% (234/247)*
Individual Categories[2]	
Healthy Donors	100% (59/59)
Healthy non-smokers	*100% (50/50)*
Healthy smokers	*100% (9/9)*
Non-GU Benign Diseases	91.7% (44/48)
Non-GU Cancer[3]	66.7% (2/3)
GU Diseases	91.9% (169/184)
BPH	*91.4% (53/58)*
Microhematuria	*86.7% (13/15)*
Interstitial Cystitis	*90.7% (10/11)*
Inflammation/Infection: Other	*100% (17/17)*
STD	*100% (2/2)*
Other	*91.4% (74/81)*
GU Cancer (non-bladder)	91.8% (56/61)
Prostate	*91.4% (53/58)*
Renal	*100% (3/3)*
GU Trauma	100% (2/2)

[1]Smoking status unknown in 1 patient.
[2]Some non-healthy patients had health conditions falling into multiple disease categories, resulting in totals >275 for individual disease categories.
[3]Non-GU cancers included breast (1), colon (1), and leukemia (1)

Based on the patient population in this study, the UroVysion test demonstrated an overall specificity of 93.0% (332/357), with a 100% specificity (59/59) among healthy patients. The specificity among unique cases was 94.5% (260/275). The false positive results found in 15 patients represented the following categories (note that some patients had health conditions falling into multiple disease categories); non-

genitourinary (GU) benign diseases (3), non-GU cancer (2), GU diseases (15), and GU cancer (5). These results indicate that the test is highly specific in this patient group, which reinforces the fact that FISH does not generate artificial aneuploidy determinations; the FISH probes react only with the intended chromosomes.

Performance vs. Standard of Care

Study Summary

A multi-center, prospective, longitudinal study was conducted to further define the performance characteristics of the UroVysion Kit relative to cystoscopy followed by histology, the standard of care for monitoring for disease recurrence in patients previously diagnosed with bladder cancer. The comparative reference used for all percent agreement calculations was cystoscopy with histology confirmation for positive or suspicious cystoscopies. If a patient had a positive cystoscopy but histology was absent (e.g., the lesion was fulgurated), then the specimen was considered positive for bladder cancer. If a test had a suspicious cystoscopy but histology was absent, then the case was omitted from analysis. A total of 309 patient visits were conducted at 21 investigation sites, resulting in 251 usable office visits. The 58 unusable visits included 17 that did not meet the eligibility criteria, 16 with insufficient urine volume, 10 with suspicious cystoscopies but no histology, and in 15 cases urine was not sent to the testing laboratories. Urine processing and analysis were conducted at one centralized testing laboratory. FISH assay and analysis on the 251 usable office visits resulted in 234 informative results, representing 176 unique patients. For patients who experienced a recurrence during the trial (as determined by cystoscopy and/or histology), the first positive visit was used (*i.e.*, the visit at which the diagnosis of recurrence was established). For the non-recurring patients, the last negative visit was used for those patients with more than one visit. The demographics for the 176 unique patients are summarized in Table 5.

Table 5
Patient Demographics

Sex	
Male	132
Female	44
Race	
Caucasian	153
African American	3
Hispanic	3
Other	13
Unknown	4
Age	
Range	36 – 98 years
Average	71 years

Technical Performance: Informative vs. Non-Informative Results
FISH assays on 70% (175/251) of the eligible study specimens were informative on the first attempt. Of the 76 specimens that failed to yield informative results on the first attempt, only 26 were due to hybridization failures. The hybridization efficiency for the first assay attempt was 87%. The remaining non-informative assays were the result of poor specimen quality (e.g., insufficient number of cells) or technical error (e.g., broken slide).

Repeat assays were conducted on 70 specimens; six of the 76 specimens had insufficient volume remaining to repeat the assay. Of the 70 repeat assays, 59 yielded informative results, leaving 17 specimens classified as "non-informative" (including the 6 cases with insufficient volume for repeat assay). In summary, over 93% of the cases yielded an informative result on the first or second attempt.

Performance vs. Standard of Care
Of the eligible patients with informative FISH results, 62 were positive by cystoscopy/histology. A breakdown of the number of tumors by stage and grade is shown in Table 6.

Table 6
Number of Tumors, by Stage and Grade

Tumor Stage	Tumor Grade					
	ND	1	2	3	Unknown	Total
ND	11	0	0	0	0	11
Ta	0	20	6	6	0	32
T1	0	0	2	3	1	6
T2	0	0	0	2	1	3
Tis	0	0	0	7	0	7
Unknown	0	2	1	0	0	3
Total	11	22	9	18	2	62

ND = not assigned or no biopsy

Table 7 shows the performance of the UroVysion Kit, relative to cystoscopy / histology, by tumor stage and grade for all cases with biopsy information available. The UroVysion Kit showed greatest agreement of positive results (100%) among the most severe tumors (T2 and Tis), when compared to cystoscopy/histology.

Table 7
Comparison of UroVysion vs. Cystoscopy/Histology for Detection of Bladder Cancer Recurrence by Tumor Stage and Grade*

	Agreement of (+) Results (%)
Stage:	
All	36/48 (75.0%)
Ta, Grade 1	11/20 (55.0%)
Ta, Grade 2,3	10/12 (83.3%)
T1	5/6 (83.3%)
T2	3/3 (100%)
Tis	7/7 (100%)
Grade:	
All	36/49 (73.5%)
1	12/22 (54.5%)
2	7/9 (77.8%)
3	17/18 (94.4%)

*Biopsy was not performed in 11 cases. In addition, no stage was assigned in 3 cases and no grade in 2.

Table 8 shows a comparison of the performance of the UroVysion Kit relative to cystoscopy followed by histology. Overall, FISH analysis with the UroVysion Kit demonstrated a percent agreement of positive results of 71.0% and a percent agreement of negative results of 65.8% when compared to the results of cystoscopy, followed by histology in the case of positive or suspicious cystoscopy (*Note*: A positive cystoscopy without a biopsy was considered positive in this analysis).

Table 8
Comparison of UroVysion vs. Cystoscopy/Histology for Detection of Bladder Cancer Recurrence

		Cysto/Histo +	Cysto/Histo -	Total
FISH	+	44	39	83
	-	18	75	93
	Total	62	114	176

Agreement of (+) results = 71.0% (95% CI = 58.1% - 81.8%)
Agreement of (-) results = 65.8% (95% CI = 56.3% - 74.4%)
Overall Agreement = 67.6% (95% CI = 60.2% - 74.5%)
(+) Predictive Value = 53.0% (95% CI = 41.7%-64.1%)
(-) Predictive Value = 80.6% (95% CI = 71.1% - 88.1%)
Prevalence = 35.2% (95% CI = 28.2% - 42.8%)
p = <0.0001 (Fisher's Exact Test)

The positive and negative predictive values of the UroVysion Test could be determined for prevalence rates of 10%, 20% and 30%; these are presented in Table 9. This extrapolation assumed a percent agreement of positive results of 71.0% and a percent agreement of negative results of 65.8% (Table 8).

Table 9
Hypothetical Positive Predictive and Negative Predictive Values of the UroVysion Test

Bladder Cancer Recurrence Prevalence	PPV	NPV
10%	18.7%	95.3%
20%	34.2%	90.1%
30%	47.1%	84.1%

Table 10 shows a comparison of the performance of the UroVysion Kit relative to cystoscopy/ histology in patients who had received their last treatment with intravesical BCG within 3 months of FISH testing. The mean time duration of BCG treatment was 1.3 months (range 0.4-3.4 months). The mean time between the last BCG treatment and FISH testing among these patients was 1.3 months; the range was 0 (treatment ongoing at the time of FISH testing) to 3 months. Three of the 12 true positive cases were Tis, three were stage Ta grade 1, three were stage Ta grade 3, two were stage T1 grade 3, and one was stage T2 grade 3 (muscle invasive); the one false negative case was stage Ta grade 1.

Table 10
Comparison of FISH vs. Cystoscopy/Histology for Detection of Bladder Cancer Recurrence in Patients on BCG Therapy within 3 Months

		Cysto/Histo +	Cysto/Histo -	Total
FISH	+	12	10	22
	-	1	16	17
	Total	13	26	39

Agreement of (+) results = 92.3% (95% CI = 64.0% - 99.8%)
Agreement of (-) results = 61.5 % (95% CI = 40.6% - 79.8%)
Overall Agreement = 71.8% (95% CI = 55.1% - 85.0%)
(+) Predictive Value = 54.5% (95% CI = 32.2% - 75.6%)
(-) Predictive Value = 94.1% (95% CI = 71.3% - 99.9%)
Prevalence = 33.3% (95% CI = 19.1% - 50.2%)
p = 0.0014 (Fisher's Exact Test)

Substantial Equivalence vs. BTAstat Test

In the clinical study described above, the performance of the UroVysion test was also compared to that of the BTA*stat* test to establish substantial equivalence of the two tests. Urine specimens from each of the 176 unique patients (first positive, last negative office visit) were also analyzed by the BTA*stat* test. Cytology was also performed on the study specimens and results are included for information purposes.

Tables 11 and 12 show the percent agreement of results of the UroVysion test, the BTA*stat* test and cytology by tumor stage and tumor grade. The UroVysion test showed greater percent agreement of positive results for all tumor stages, including 100% agreement for T2 and Tis tumors.

Table 11
Percent Agreement of (+) Results Analysis by Tumor Stage

		Ta, 1 – Total: 20 Cases		
55.0%	FISH	11 Positive	9 Negative	
20.0%	Cytology	4 Positive	16 Negative	
30.0%	BTA*stat*	6 Positive	14 Negative	
		Ta 2,3 – Total: 12 Cases		
83.3%	FISH	10 Positive	2 Negative	
33.3%	Cytology	4 Positive	8 Negative	
83.3%	BTA*stat*	10 Positive	2 Negative	
		T1 – Total: 6 Cases		
83.3%	FISH	5 Positive	1 Negative	
66.7%	Cytology	4 Positive	2 Negative	
83.3%	BTA*stat*	5 Positive	1 Negative	
		T2 – Total: 3 Cases		
100%	FISH	3 Positive	0 Negative	
33.3%	Cytology	1 Positive	2 Negative	
66.7%	BTA*stat*	2 Positive	1 Negative	
		Tis – Total: 7 Cases		
100%	FISH	7 Positive	0 Negative	
33.3%	Cytology	2 Positive	4 Negative	1 inconclusive
42.9%	BTA*stat*	3 Positive	4 Negative	

NOTE: Three (3) cases were considered Unknown by Central Pathology for Tumor Stage

Table 12
Percent Agreement of (+) Results Analysis by Tumor Grade

Grade 1 – Total: 22 Cases				
54.5%	FISH	12 Positive	10 Negative	
18.2%	Cytology	4 Positive	18 Negative	
27.3%	BTA*stat*	6 Positive	16 Negative	
Grade 2 – Total: 9 Cases				
77.8%	FISH	7 Positive	2 Negative	
44.4%	Cytology	4 Positive	5 Negative	
77.8%	BTA*stat*	7 Positive	2 Negative	
Grade 3 – Total: 18 Cases				
94.4%	FISH	17 Positive	1 Negative	
41.2%	Cytology	7 Positive	10 Negative	1 inconclusive
72.2%	BTA*stat*	13 Positive	5 Negative	

NOTE: Two (2) cases were considered Unknown by Central Pathology for Tumor Grade.

Table 13 shows a comparison of the performance of the BTA*stat* test relative to cystoscopy/histology among the unique patients (first positive, last negative office visit). Overall, analysis with the BTA*stat* test demonstrated a percent agreement of positive results of 50.0% and a percent agreement of negative results of 69.3% when compared to the results of cystoscopy followed by histology in the case of positive or suspicious cystoscopy. (*Note*: A positive cystoscopy without a biopsy was considered positive in this analysis). In a comparison of the UroVysion Kit with cystoscopy/ histology on the same dataset (Table 8), the UroVysion Kit showed a percent agreement of positive results of 71.0% and a percent agreement of negative results of 65.8% (Table 8).

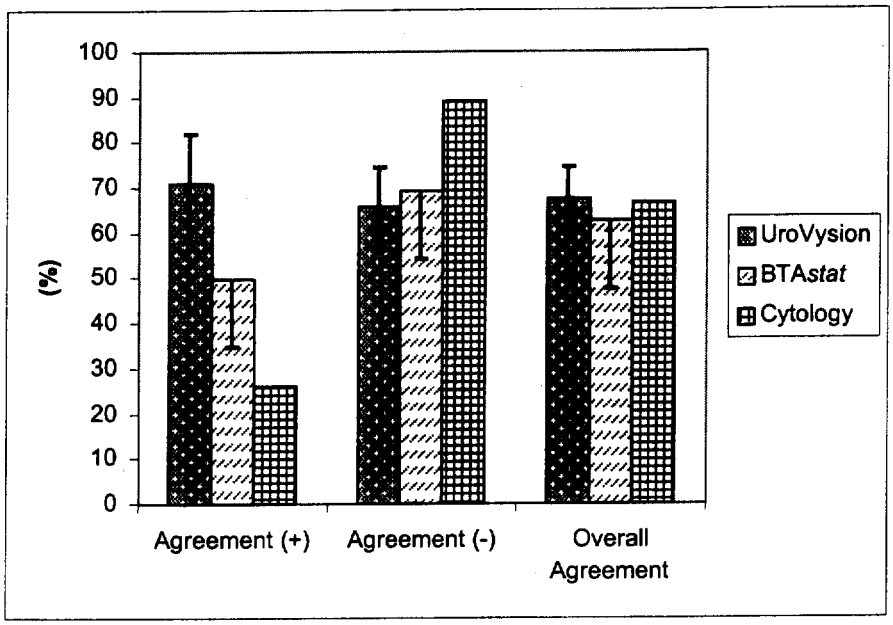
Table 13
Comparison of BTA*stat* vs. Cystoscopy/Histology for Detection of Bladder Cancer Recurrence

BTA*stat* \ Cysto/Histo	+	-	Total
+	31	35	66
-	31	79	110
Total	62	114	176

Agreement of (+) results = 50.0% (95% CI = 37.0% - 63.0%)
Agreement of (-) results = 69.3% (95% CI = 60.0% - 77.6%)
Overall Agreement = 62.5% (95% CI = 54.9% - 69.7%)
(+) Predictive Value = 47.0% (95% CI = 34.6% - 59.7%)
(-) Predictive Value = 71.8% (95% CI = 62.4% - 80.0%)
Prevalence = 35.2% (95% CI = 28.2% - 42.8%)

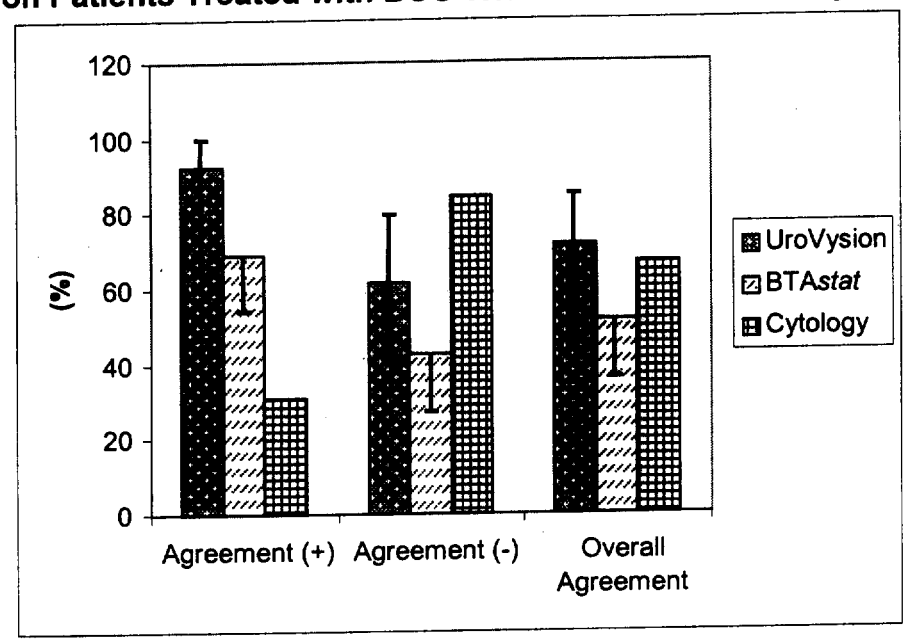
Figure 1 compares the percent agreement of results for FISH, BTA*stat* and cytology (unique patient visits), relative to cystoscopy/histology. The UroVysion test's two tail lower 95% CI for percent agreement of positive, negative and overall results was 58.1%. 56.3% and 60.2%, respectively. On the corresponding dataset assayed with the BTA*stat* test, the scores minus 15% were 35.0%, 54.3% and 47.5%, respectively. Thus, the criteria for substantial equivalence of the UroVysion assay to the BTA*stat* test were met; the 95% CIs for UroVysion are greater than the BTA*stat* scores minus 15%. This is represented graphically in Figure 1; the error bars represent the upper and lower 95% CIs for the UroVysion test results and the test score minus 15% for the BTA*stat* test results. Again, as shown in the figure, in each case the 95% CI for UroVysion is greater than the BTA*stat* score minus 15%.

Figure 1
Comparison of Three Detection Methods Relative to Cystoscopy/Histology



A summary of the percent agreement of the three detection methods in the group of patients treated with BCG within the last 3 months is shown in Figure 2 (unique patient visits). In this group, the UroVysion test's two tail lower 95% CI for percent agreement of positive, negative and overall results was 64.0%. 40.6% and 55.1%, respectively. On the corresponding dataset assayed with the BTA*stat* test, the scores minus 15% were 54.2%, 27.3% and 36.3%, respectively. Thus, the criteria for substantial equivalence of the UroVysion assay to the BTA*stat* test were met; the 95% CIs for UroVysion are greater than the BTA*stat* scores minus 15%. This is represented graphically in Figure 2; the error bars represent the upper and lower 95% CIs for the UroVysion test results and the test score minus 15% for the BTA*stat* test results. Again, as shown in the figure, in each case the 95% CI for UroVysion is greater than the BTA*stat* score minus 15%.

Figure 2
Comparison of Three Detection Methods Relative to Cystoscopy/Histology on Patients Treated with BCG Within 3 Months of Study Visit



The UroVysion test and the BTA*stat* test were each compared to cytology on patients positive for recurrence, as determined by cystoscopy/histology; the results are shown in Tables 14 and 15. Cytology did not pick up any cases that were negative by FISH (Table 14). Cytology was positive in 2 cases found negative by BTA*stat* (Table 15).

Table 14
Comparison of FISH vs. Cytology Results in Patients Positive for Recurrence

		Cytology		
		+	-	Total
FISH	+	16	27	43
	-	0	18	18
	Total	16	45	61

Note: One (1) Tis case was scored inconclusive for cytology and not included in this table.

Table 15
Comparison of BTA*stat* vs. Cytology Results in Patients Positive for Recurrence

		Cytology		
		+	-	Total
BTA*stat*	+	15	16	31
	-	1	29	30
	Total	16	45	61

Note: One (1) Tis case was scored inconclusive for cytology and not included in this table.

The results for the percent agreement of results for the UroVysion test (FISH), the BTA*stat* test and cytology are summarized in Table 16 (per patient office visit).

Table 16
Summary: Methods Comparison

		FISH	BTA*stat*	Cytology
Overall	Agreement of (+) Results	71.0%	50.0%	26.2%
	Agreement of (-) Results	65.8%	69.3%	89.1%
	Overall Agreement	67.6%	62.5%	66.7%
BCG Treatment	Agreement of (+) Results	92.3%	69.2%	30.8%
	Agreement of (-) Results	61.5%	42.3%	84.6%
	Overall Agreement	71.8%	51.3%	66.7%

Table 17 shows a head-to-head comparison of the results from the UroVysion test and the BTA*stat* test on those cases (unique office visits) with informative results for both tests. The concordance between the two tests was 61.9%.

Table 17
Concordance of FISH vs. BTA*stat*

		BTA*stat* +	BTA*stat* -	Total
FISH	+	41	42	83
	-	25	68	93
	Total	66	110	176

An analysis of the discordant results is presented in Table 18. Of the cases positive by FISH and negative by BTA*stat*, 21 (50.0%) were positive by either cytology or cystoscopy/histology, or both, including 4 Tis tumors and 1 T2 tumor (Table 18). Of the 25 cases negative by FISH and positive by BTA*stat*, only 3 (12.0%) were positive by one or both of the comparative methods.

Table 18
FISH vs. BTA*stat*: Discordant Analysis

	FISH "+" / BTA*stat* "-"	FISH "-" / BTA*stat* "+"
N	42	25
Cytology "+"	7 (16.7%)	1 (4.0%)
Cysto/Histo "+"	15 (35.7%)	2 (8.0%)
Ta	6	1
T1	--	--
T2	1	--
Tis	4	--
Unk	1	1
Pos/No Biopsy	3	--
Cytology "+" or Cysto/Histo "+"	21 (50.0%)	3 (12.0%)

Note: One (1) case showed both positive cytology and positive cystoscopy/histology in the FISH "+"/BTA*stat* "-" group.

HYBrite/VP 2000 Validation

The VP2000 is considered to be a class I, exempt device according to **21 CFR § 864.3800 Automated slide stainer**, and **21 CFR § 864.3875 Automated tissue processor.** The function of the VP2000 is consistent with both of the above paragraphs from the CFR. Indeed, except for minor modifications the device is exactly the same device as the custom OEM device bought and sold by Zeiss during the past decade as a class I device for cytology laboratories.

The paragraphs from the CFR are reproduced below:

> **21 CFR § 864.3800 Automated slide stainer.** (a) *Identification.* An automated slide stainer is a device used to stain histology, cytology and hematology slides for diagnosis. (b) *Classification.* Class I. The device is exempt from the premarket notification procedures in Subpart E of Part 807 of this chapter.
>
> **21 CFR § 864.3875 Automated tissue processor.** (a) *Identification.* An automated tissue processor is an automated system used to process tissue specimens for examination through fixation, dehydration, and infiltration. (b) *Classification.* Class I. The device is exempt from the premarket notification procedures in Subpart E of Part 807 of this chapter.

A validation study was conducted to determine if the recommended specimen pretreatment protocol and assay for the UroVysion Kit performed the same whether done manually by technician or by semi-automated using the VP2000 Sample Processor and HYBrite instruments.

Study specimens consisted of three human urine pools prepared from voided urine specimens obtained from normal donors. Study specimens used in the Assay Interference Study, Protocol 99-402R (see Appendix B for protocol and study report) were also used as part of this study. Each of the 29 substances which were spiked into aliquots of each of the three pools at two different concentrations were tested on three separate VP-2000 and HYBrite instruments and compared to results obtained in the manual study.

Quality evaluations from samples of the 23 different compounds and 6 preservatives tested produced equivalent results using the UroVysion Kit and FISH Pretreatment Kit for all concentrations tested and across all three instrument set-ups.

Normal urine pools (unspiked) and manual assay results from the Interference Study Protocol, 99-402R were used as controls. All compounds and preservatives identified in Table 19 performed within 2 standard deviations or 20% of the control pools, supporting the conclusion that the manual and semi-automated methods are equivalent.

Table 19
Manual versus Semi-Automation Study Results

Substance	Concentrations	Results- Manual vs Semi-Automation
Possible Urine Constituents		
Albumin	0.5 g/dL and 1.0 g/dL	Equivalent.
Ascorbic Acid	2.5 g/dL and 5 g/dL	Equivalent.
Bilirubin (unconjugated)	1 mg/mL and 2 mg/mL	Equivalent.
Hemoglobin	50 mg/mL and 100 mg/mL	Equivalent.
IgG	5 mg/dL and 10 mg/dL	Equivalent.
Red Blood Cells (human)	5×10^5 cells/mL and 1×10^6 cells/mL	Equivalent.
White Blood Cells (human)	5×10^5 cels/mL and 1×10^6 cells/mL	Equivalent.
Sodium Chloride	365 mg/dL and 730 mg/dL	Equivalent.
Uric Acid	125 mg/dL and 250 mg/dL	Equivalent.
Caffeine	58.5 mg/dL and 117 mg/dL	Equivalent.
Ethanol	0.5% (v/v) and 1% (v/v)	Equivalent.
Nicotine	14 mg/dL and 28 mg/dL	Equivalent.
Possible Microbial Contaminants		
Candida albicans	1.25×10^{10} CFU/mL and 2.5×10^{10} CFU/mL	Equivalent.
Escherichia coli	1.25×10^{10} CFU/mL and 2.5×10^{10} CFU/mL	Equivalent.
Pseudomonas aerugenosa	1.25×10^{10} CFU/mL and 2.5×10^{12} CFU/mL	Equivalent.
Therapeutic Agents		
Acetaminophen	2.6 g/dL and 5.2 g/dL	Equivalent.
Acetylsalicylic Acid	2.6 g/dL and 5.2 g/dL	Equivalent.
Ampicillin	300 mg/dL and 600 mg/dL	Equivalent.
BCG	10 mg/dL and 20 mg/dL	Equivalent.
Doxorubicin-HCl	5 mg/dL and 10 mg/dL	Equivalent.
Mitomycin C	5 mg/dL and 10 mg/dL	Equivalent.
Nitrofurantoin	25 mg/dL and 50 mg/dL	Equivalent.
Phenazopyridine-HCl	100 mg/dL and 200 mg/dL	Equivalent.
Thiotepa	5 mg/dL and 10 mg/dL	Equivalent.
Trimethoprin	25 mg/dL and 50 mg/dL	Equivalent.
Preservatives		
Vysis, Inc. standard: 2% Carbowax	2% Carbowax/50% ethanol solution (33 ml urine with 17 mL preservative)	Equivalent.
UroCor, Inc. fixative	50/50 with urine	Equivalent.
CytRichRed (Autocyte)	50/50 with urine	Equivalent.
Saccamono's solution	50/50 with urine	Equivalent.
PreservCyt solution (Cytyc)	50/50 with urine	Equivalent.
100% Ethanol	50/50 with urine	Equivalent.

Conclusions

The clinical studies described in this document demonstrate that the performance of UroVysion Kit is safe and effective. The performance of the UroVysion Kit is also supported by the Vysis Quality Control procedures. When the UroVysion Kit is used as instructed in the package insert, the above statements describe its performance.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

AUG - 3 2001

Russel K. Enns, Ph.D.
Vice President of Regulatory Affairs
Vysis, Inc.
3100 Woodcreek Drive
Downers Grove, Illinois 60515

Re: K011031
Trade Name: Vysis™ Inc., UroVysion™ Bladder Cancer Recurrence Kit
Regulation Number: 21 CFR § 866.6010
Regulatory Class: II
Product Code: MMW
Dated: May 30, 2001
Received: May 31, 2001

Dear Dr. Enns:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the Current Good Manufacturing Practice requirements, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic QS inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal laws or regulations.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its internet address "http://www.fda.gov/cdrh/dsma/dsmamain.html".

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical Laboratory Devices
Office of Device Evaluation
Center for Devices and Radiological Health

Enclosure

510(k) Number (IF KNOWN): K011031

DEVICE NAME: Vysis™, Inc. UroVysion™ Bladder Cancer Recurrence Kit

INDICATIONS FOR USE:

The UroVysion Bladder Cancer Recurrence Kit (UroVysion Kit) is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence *in situ* hybridization (FISH) in urine specimens from subjects with transitional cell carcinoma of the bladder. Results from the UroVysion Kit are intended for use as a noninvasive method for monitoring for tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with bladder cancer.

(Division Sign-Off)
Division of Clinical Laboratory Devices

510(k) Number K011031

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓ OR Over-The-Counter-Use___
(Per 21 CFR 801.109) (Optimal Format 1-2-96)